PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Shea N. Gardner et al	Examiner: Angela Bertagna
Serial No.: 10/727,779	Art Unit: 1637
Filed: 12/03/2003	Attorney Docket: IL-11191
TITLE: SEQUENTIAL ADDITION OF SHORT DNA OLIGOSIN DNA-POLYMERASE-BASED SYNTHESIS REACTIONS	

Honorable Commissioner for Patents
Alexandria, VA 22313-1450

Attention: Board of Patent Appeals and Interferences

Dear Sir:

APPELLANTS' BRIEF (37 C.F.R. § 1.192)

This brief is submitted in support of Appellants' Notice of Appeal from the Final Rejection mailed September 26, 2008 rejecting claims 11, 16, and 17.

Appellants' Notice of Appeal was filed December 16, 2008.

One copy of the brief is being transmitted per 37 C.F.R. § 41.37.

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I. REAL PARTY IN INTEREST

The real party in interest is:

Lawrence Livermore National Security, LLC and the United States of America as represented by the United States Department of Energy (DOE) by virtue of an assignment by the inventor as duly recorded in the Assignment Branch of the U.S. Patent and Trademark Office.

II. RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences.

III. STATUS OF CLAIMS

The application as originally filed contained claims 1-17.

The claims on appeal are claims 11, 16, and 17.

The status of all the claims in the proceeding (*e.g.*, rejected, allowed or confirmed, withdrawn, objected to, canceled) is:

Claims 1-10 are withdrawn.

Claims 12-15 have been cancelled.

Claims 11, 16, and 17 are rejected.

Claims 11, 16, and 17 on appeal are reproduced in the Appendix.

IV. STATUS OF AMENDMENTS

There have been no amendments filed subsequent to the Final Rejection mailed September 26, 2008.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Appellants' claimed invention is illustrated FIG. 4 reproduced below and is described in the portions of Appellants' specification quoted below with the quoted information identified by page and line numbers.

[0007] The present invention provides a method of fabricating a DNA molecule of user-defined sequence, comprising the steps of preselecting a multiplicity of DNA sequence segments that will comprise the DNA molecule of user-defined sequence, separating the DNA sequence segments temporally, and combining the multiplicity of DNA sequence segments with at least one polymerase enzyme wherein the multiplicity of DNA sequence segments join to produce the DNA molecule of user-defined sequence. The method comprising preselecting a multiplicity of DNA sequence segments that will comprise the DNA molecule using computational techniques to break the user-defined sequence into fragments of defined size, arraying the fragments of defined size into groups, and assembling the groups into double-strand DNA molecules of predetermined base-pairs to produce the DNA molecule of user-defined sequence. In one embodiment the step of separating the DNA sequence segments temporally is accomplished by the DNA sequence segments being added gradually, in sequence order. In another embodiment the step of separating the DNA sequence segments temporally is accomplished by the DNA sequence segments being added gradually, in an order that is predicted computationally to minimize errors. In another embodiment a method of fabricating a DNA molecule of user-defined sequence comprises preselecting a multiplicity of DNA sequence segments that will comprise the DNA molecule of user-defined sequence, and combining the multiplicity of DNA sequence segments with at least one polymerase enzyme wherein the multiplicity of DNA sequence segments

join to produce the DNA molecule of user-defined sequence. (Page 4, lines 5-25 and Page 5, lines 1-2)

[0040] Description of Another Embodiment – Applicants have previously described various embodiment of the invention comprising fabricating a DNA molecule of user-defined sequence by preselecting a multiplicity of DNA sequence segments that will comprise the DNA molecule of user-defined sequence, wherein the multiplicity of DNA sequence segments comprise n-mers, wherein n is a number less than 20, and combining the multiplicity of DNA sequence segments with at least one polymerase enzyme wherein the multiplicity of DNA sequence segments join to produce the DNA molecule of user-defined sequence. This embodiment provides an example of the polymerase-based synthesis of a user-defined DNA sequence, using pre-synthesized 8-base, single-stranded DNA molecules (8-mers) as starting substrates. For purposes of this example, when DNA is described as 40 bases long, it is presumed to be single-stranded. When DNA is described as being 40 base pairs, it is presumed to be double-stranded. (Page 17, lines 3-15)

[0041] How a sequence is chosen: The DNA sequence is entirely user-defined. It can be a specific gene, human or otherwise, that one wishes to synthesize. It can also be a purely arbitrary DNA sequence, of any length. This DNA sequence will be made using very short oligonucleotides (synthetic single-stranded DNA molecules) and DNA polymerase. (Page 17, lines 16-20)

[0042] Why a sequence is divided into short oligos: Once the sequence to be synthesized has been chosen, it is divided into 8-base segments, since these are the components from which the final product will be assembled. As the final DNA molecule will be double-stranded, for any given length of DNA, (e.g., 40 base pairs) the number of 8-mers necessary to synthesize it must be sufficient to produce a double-stranded molecule. Thus, rather than five 8-mers to make a 40- base-pair final product (8x5=40), the synthesis would require ten 8-mers. Once the desired molecule has been divided, the appropriate 8-mers can be gathered (from the possible 65,536 8-mers) for the subsequent polymerase-based assembly. (Page 17, lines 21-25 and Page 18, lines 1-4)

[0043] Why parallel synthesis is so important: There are literally billions of possible sequences resulting from combining the thousands of 8-mers

necessary to make a gene-length product. If all of the component 8-mers were combined and reacted with DNA polymerase, the result would be a random collection of DNA, of innumerable different sequences, possibly none representing the correct one. It is therefore necessary to combine the component 8-mers into smaller groups, as this is the method most likely to minimize errors while producing the maximum amount of the correct product. The groups into which the 8-mers are divided will contain highly variable numbers of 8-mers; the size of each group is dependent on the initial sequence of the desired product. To make, for example, an 800-base-pair DNA molecule requires two hundred 8-mers. These may be combined in groups of any number from two to 200, depending on the sequence of the 800-base-pair product. The standard for the groups is ten 8-mers, or enough to produce a 40-base-pair DNA molecule. (Page 18, lines 5-18)

[0044] How a sequence is divided into short oligos: The desired final sequence is divided into its component 8-mers by a computer program. This program uses the thermodynamic and kinetic aspects of DNA base pairing to divide the final product into its precursor 8-mers. This program compares all of the possible hybridizations (the act of two single-stranded oligonucleotides joining by hydrogen bond-mediated base pairing to become a double-stranded DNA molecule) of the 8-mers into which the target DNA molecule has been divided. There are many opportunities for error in the synthesis process, most of which are caused by improper hybridization of 8-mers to one another. The computer program is designed to minimize these errors by combining the 8-mers into small groups that are the most likely to produce the desired product, and the least likely to produce errors. (Page 18, lines 19-26 and Page 19, lines 1-4)

[0045] How the short oligos are assembled into longer DNA molecules: The groups of 8-mers are combined with the necessary reagents to allow DNA polymerase-based DNA synthesis. These include the appropriate buffers and nucleotides. The specifics of this process are contained in the 4-mer embodiment. Once the 8-mers have been converted into the desired product (e.g., 40-base-pair molecules), then these 40 base-pair products are themselves combined into subsequent polymerase-mediated reactions, in combinations dictated by the computer program. In this way, the reactions progress from converting groups of ten 8-mers into 40-base-pair molecules, then converting groups of 40 base-pair molecules into longer (e.g., 200-base-

pair) molecules, and then finally converting these into the final, desired product. (Page 19, lines 5-15)

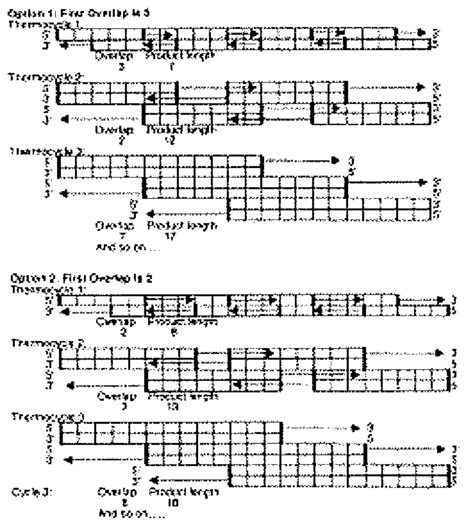

FIG.4

[0046] Section 1: Use of odd-sized starting oligos - Referring now to FIG. 4, another embodiment of a system of creating long DNA sequences, e.g., 1-10 kilobases, from short oligos of length n (n-mers) of the present invention is illustrated. The system is designated generally by the reference numeral 400. The system of parallel synthesis 400 provides a process for making very long (greater than is possible with conventional phosphoramidite chemistry) DNA of user-defined sequence. The method begins by using computational techniques to break the desired sequence into fragments of defined size. (Page 19, lines 16-23)

[0047] These n-base fragments are then arrayed in groups of n-base oligonucleotides and assembled into double-strand DNA molecules using DNA polymerase. The starting oligos may be of size n, where n is an odd number. The desired, hybridizing overlaps between oligos in the first thermocycle of PCR may be specified by the user. Table 1 gives a few examples of the overlap length, oligo length, and number of polymerized bases for several scenarios of starting oligo size and overlap in the first thermocycle, and the formula for computing these variables. The products of the reactions in the first tier of PCR reactions (each PCR reaction involves many thermocycles) are then combined, in as many steps as necessary, and assembled by polymerase into still-longer molecules, until the final desired product is assembled. The final product is then amplified using PCR. (Page 19, lines 24-26 and Page 20, lines 1-9)

[0048] The assembly process is substantially the same as the process called DNA shuffling. It is similar to PCR in that there is a template, a primer, a DNA polymerase, and the attendant nucleotides and buffers. It is dissimilar to PCR in that the primer and template are the same entities – the n-mers themselves. Following the parallel assembly process, the final product can be amplified by PCR. Any DNA polymerase commonly used for PCR can be used for this purpose. (Page 20, lines 10-16)

[0049] The system 400 is similar to the system 300 described above and illustrated in FIG. 3; however, in the system 400, the starting oligos may be of odd length instead of even length. That is, in the system 300, the oligos, or n-mers, are of even length equal to n with a hybridizing overlap between complementary oligos of length n/2 in the first two thermocycles. In contrast, in the system 400, the length n may be odd, and the overlap length between hybridizing oligos may be specified by the user. Given a desired overlap v_1 in the first thermocycle and the length n of the starting oligos that are specified by the user, the length l_c of oligonucleotides starting thermocycle c is computed by the formula: $l_c=n(c-1)+p_1$ for $c>1$, where $p_1=n-v_1$. The length v_c of desired overlap between oligos in thermocycle c is given by $v_c=n(c-2)+p_1$ for $c>1$. The number p_c of bases polymerized in thermocycle c is $p_c= n$ for $c>1$. (Page 20, lines 17-26 and Page 21, lines 1-2)

[0050] Figure 4 illustrates the first three thermocycles for the two scenarios starting with n=5 outlined in Table 1 below. Each yellow box indicates a nucleotide, and a series of yellow boxes represents an oligonucleotide, where the heavy black vertical lines indicate the ends of an oligonucleotide. The 5' and 3' ends of the plus and minus strands are labeled, and where nucleotides are in the same column (overlap vertically) and in the right orientation (5' to 3' on the top strand, and 3' to 5' on the bottom, from left to right), the desired hybridization occurs. Red arrows indicate polymerization (both the direction and the number of polymerized bases) from 3' ends during the specified thermocycle. In the first case, in which the first overlap $v_1=3$, polymerization extends each oligonucleotide by $p_1=2$ bases, and the length of the oligonucleotides starting the second thermocycle is 7 bases. In the second case, the first overlap $v_1=2$, polymerization extends each oligonucleotide by $p_1=3$ bases, and the length of the oligonucleotides starting the second thermocycle is 8 bases. These are merely two examples, and any

other values of n and v_1 specified by the user may be used. (Page 21, lines 3-17)

Appellants' single independent claim on appeal, claim 11, is "read on" Appellants' specification with the page and line numbers identified.

Claim 11	Specification & Drawings
A method of producing a DNA molecule of 1-10 kilobases of user-defined sequence from short oligos of length n (n-mers), comprising the steps of:	Referring now to FIG. 4, another embodiment of a system of creating long DNA sequences, e.g., 1-10 kilobases, from short oligos of length n (n-mers) (Page 19, lines 17-19)
virtually preselecting a multiplicity of DNA sequence segments that will comprise said DNA molecule of user-defined sequence by using computational techniques to virtually break said user-defined sequence into virtual fragments of length n (n-mers) of defined size,	preselecting a multiplicity of DNA sequence segments that will comprise the DNA molecule using computational techniques to break the user-defined sequence into fragments of defined size, (Page 4, lines 12-15) FIG. 4, … from short oligos of length n (n-mers) (Page 19, lines 16-18)
providing fragments in vitro by providing fragments of length n (n-mers) of defined size that correspond to said virtual fragments,	a system of creating long DNA sequences, e.g., 1-10 kilobases, from short oligos of length n (n-mers) (Page 19, lines 16-18)
arraying fragments in vitro by arraying said fragments of length n (n-mers) of defined size into groups,	arraying the fragments of defined size into groups, (Page 4, line 15)

Claim 11 (Continued)	Specification & Drawings
separating DNA sequence segments temporally in vitro by separating said DNA sequence segments of length n (n-mers) of defined size temporally, and	Referring now to FIG. 4, … creating long DNA sequences, e.g., 1-10 kilobases, from short oligos of length n (n-mers) of the present invention is illustrated. (Page 19, lines 16-
assembling groups in vitro by assembling said groups into double-strand DNA molecules of predetermined base-pairs using parallel synthesis, DNA shuffling, and DNA polymerase wherein said step of separating said DNA sequence segments temporally and said step of assembling said groups into double-strand DNA molecules of predetermined base-pairs is accomplished by said DNA sequence segments being added gradually, in an order that is predicted computationally to minimize errors to produce said DNA molecule of user-defined sequence, and	These n-base fragments are then arrayed in groups of n-base oligonucleotides and assembled into double-strand DNA molecules using DNA polymerase. The groups of oligos are separated temporally. That is, oligos are added gradually, in sequence order or other order that is predicted (computationally) to minimize errors, over time through the many thermocycles of hybridization and polymerization. (Page 23, lines 5-10)
wherein said step or assembling said groups into double-strand DNA molecules utilizes starting oligos of length n (n-mers) where n is an odd number.	in the system 400, the starting oligos may be of odd length instead of even length. (Page 20, Lines 18-19)

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The Final Rejection mailed September 26, 2008 states five (5) grounds of rejection. The five grounds of rejection are summarized as follows:

Grounds of Rejection #1 – Claim 16 was rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The rejection is stated in numbered paragraph 3 on pages 3-4 of the Final Rejection mailed September 26, 2008.

Grounds of Rejection #2

Claim 11 was rejected under 35 U.S.C. § 102(e) as being anticipated by Evans U.S. Published Patent Application No. 2003/0087238 (hereinafter "Evans"). The rejection is stated in numbered paragraph 5 on pages 5-6 of the Final Rejection mailed September 26, 2008.

Grounds of Rejection #3

Claims 16 and 17 were rejected under 35 U.S.C. § 102(a) and 102(e) as being anticipated by Evans. The rejection is stated in numbered paragraph 6 on page 6 of the Final Rejection mailed September 26, 2008.

Grounds of Rejection #4

Claims 11 and 17 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Selifonov et al International Patent No. WO 00/42560

(hereinafter "Selifonov") in view of Evans. The rejection is stated in numbered paragraph 8 on pages 7-10 of the Final Rejection mailed September 26, 2008.

Grounds of Rejection #5

Claim 16 was rejected under 35 U.S.C. § 103(a) as being unpatentable over Selifonov in view of Evans and further in view of Murphy et al U.S. Patent No. 6,994,963 (hereinafter "Murphy"). The rejection is stated in numbered paragraph 9 on pages 10-11 of the Final Rejection mailed September 26, 2008.

VII. ARGUMENT

Argument Relating to Grounds of Rejection #1 - The rejection in Grounds of Rejection #1 is respectfully traversed because claim 16 complies with the written description requirement of 35 U.S.C. § 112, first paragraph. The Final Rejection mailed September 26, 2008 contains the following statements:

> "Claim 16 is drawn to the method of claim 11, wherein the starting oligos of length n (n-triers), where n is an odd number, have a length of n+1 or n+2. Claim 16 as originally filed required the starting oligos to have a length n+l, n+2, etc. Therefore, the amendment to claim 16 broadens the scope of the claims by only requiring the starting oligos to have a length of n+1 or n+2 rather than lengths of n+1 and n+2, etc."

"Applicant's response does not indicate where the amendment finds support in the original disclosure. The specification teaches in paragraphs 63 and 64 that the starting oligos have a length of n+1, n+2, etc, but does not provide support for a broader embodiment of the method wherein starting oligos having a length of n+1 or n+2, where n is an odd number, are used to practice the method of claim 11. Therefore, the method of amended claim 16 is not adequately supported by the original disclosure, and it has been rejected under 35 U.S.C. § 112, first paragraph for incorporating new matter."

Appellants' respectfully traverse these statements because there is support for the term "starting oligos of length n+1 or n+2" in Appellants' original specification and drawings. Claim 16 is reproduced below for reference.

Claim 16. The method of producing a DNA molecule of user-defined sequence of claim 11 wherein said starting oligos of length n (n-mers) where n is an odd number are starting oligos of length n+1 or n+2.

Appellants' original specification and drawing figure 4 reproduced below include the following support for the term "starting oligos of length n+1 or n+2" used in claim 16.

"[0009] ... FIG. 7 illustrates another embodiment of a system of creating long DNA sequences, e.g., 1-10 kilobases, from short oligos of length n, n+1, n+2, etc. of the present invention." (Page 6, lines 4-6 of Appellants' Original Specification)

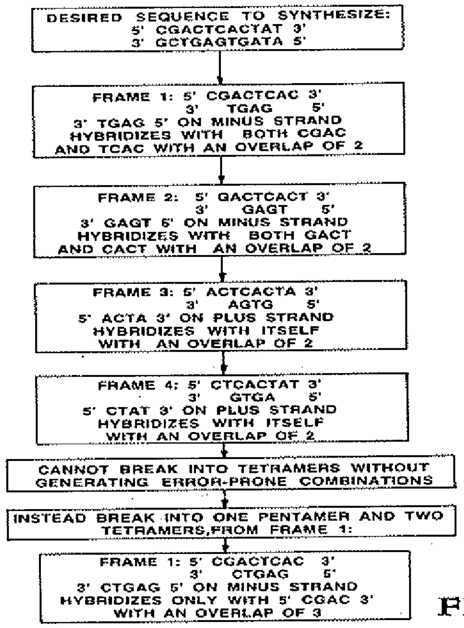

FIG. 7

"[0063] … Referring now to FIG. 7, another embodiment of a system of creating long DNA sequences, e.g., 1-10 kilobases, from short oligos of length n, n+1, n+2, etc. of the present invention is illustrated." (Page 27, lines 13-15 of Appellants' Original Specification)

"[0064] These fragments of multiple sizes are then arrayed in groups of oligonucleotides and assembled into double-strand DNA molecules using DNA polymerase. The starting oligos may be of size n, n+1, n+2, etc." (Page 28, lines 3-5 of Appellants' Original Specification)

Appellants' point out that there would be no question of support if claim 16 used the term "starting oligos of length n+1." Similarly, there would be no question of support if claim 16 used the term "starting oligos of length n+2." Therefore there should be no question of support when claim 16 uses the term "starting oligos of length n+1 or n+2."

The law of patent claiming allows use of the term "starting oligos of length n+1 or n+2" when the examples provided in the patent application disclose enumerated elements "n+1" and "n+2." Appellants' could use the term "starting oligos of length n+1" in a claim and the claim would be supported by the application which discloses enumerated elements "n+1" and "n+2." Appellants' could also use the term "starting oligos of length n+2" in a claim and the claim would be supported by the application which discloses enumerated elements "n+1" and "n+2." Therefore the law of patent claiming allows use of the term "starting oligos of length n+1 or n+2" when the examples provided in the patent application disclose enumerated elements "n+1" and "n+2."

Appellants' claim 16 complies with the written description requirement of 35 U.S.C. § 112, first paragraph, because there is support in Appellants' original specification for the terms used in claim 16. It is respectfully requested that the rejection of claim 16 in Grounds of Rejection #1 be reversed.

Argument Relating to Grounds of Rejection #2 - The rejection in Grounds of Rejection #2 is respectfully traversed because claim 11 is not anticipated by the Evans reference.

The Evans Reference

The Evans reference is United States Published Patent Application No. 2003/0087238 for a method for assembly of a polynucleotide encoding a target polypeptide. The system of the Evans reference utilizes the results of genomic sequence information by computer-directed polynucleotide assembly based upon information available in databases such as the human genome database. The system is illustrated in Figure 5 from the Evans reference reproduced below and is described in the specification of the Evans reference quoted below.

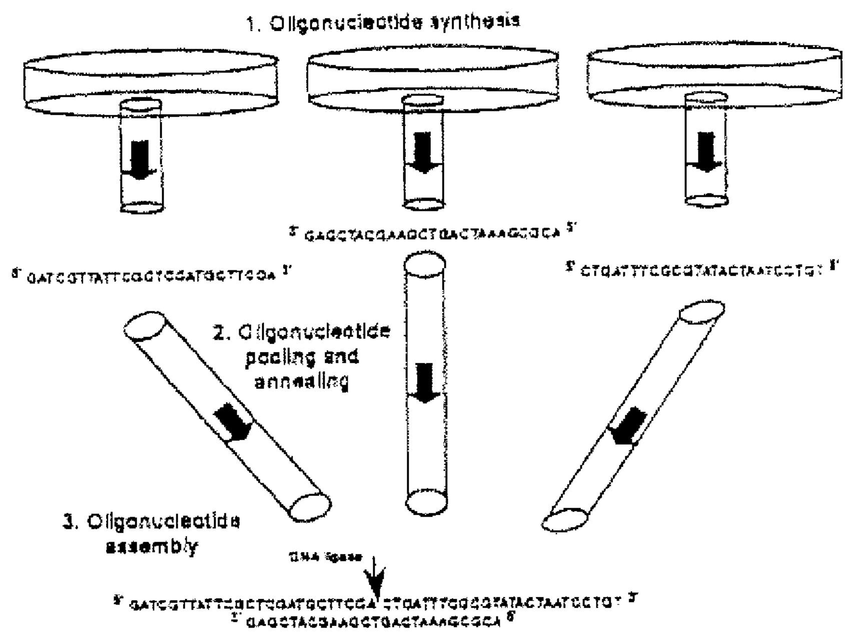

Figure 5

"[0012] FIG. 5 depicts that oligonucleotide synthesis, oligonucleotide assembly by pooling and annealing, and ligation can be accomplished using microfluidic mixing."

"[0180] As shown in FIG. 5, oligonucleotide synthesis, oligonucleotide assembly by pooling and annealing, and ligation can be done using microfluidic mixing, resulting in the same set of critical triplex intermediates that serves as the substrate for annealing, ligation and oligonucleotide joining. DNA ligase and other components can be placed in the buffer fluid moving through the instrument microchambers. Thus, synthesis and assembly can be carried out in a highly controlled way in the same instrument."

<u>The Evans Reference Does Not Anticipate Claim 11</u>

Appellants will show that Appellant's claim 11 is not anticipated by the Evans reference. The standard for a 35 U.S.C. § 102 rejection is stated in *RCA Corp. v. Applied Digital Systems, Inc,* 221PQ 385, 388 (d. Cir. 1984) "Anticipation is established only when a single prior art reference discloses, either expressly or under principles of inherency, each and every element of a claimed invention." Appellants point out that the following elements of Appellant's claim 11 are not found in the Evans reference:

> "assembling groups in vitro by assembling said groups into double-strand DNA molecules of predetermined base-pairs using parallel synthesis, DNA shuffling, and DNA polymerase wherein said step of separating said DNA sequence segments temporally and said step of assembling said groups into double-strand DNA molecules of predetermined base-pairs is accomplished by said DNA sequence segments being added gradually, in an order that is predicted computationally to minimize errors to produce said DNA molecule of user-defined sequence," or
> "wherein said step or assembling said groups into double-strand DNA molecules utilizes starting oligos of length n (n-mers) where n is an odd number."

Since the elements described above are not found in the Evans reference, the Evans reference does not support a 35 U.S.C. § 102(b) rejection of Appellant's claim 11 and the rejection in Grounds of Rejection #2 should be reversed.

Appellants point out that the following combination of claim elements specified in Appellant's claim 11 are not shown by the Evans reference:

> "virtually preselecting a multiplicity of DNA sequence segments that will comprise said DNA molecule of user-defined sequence by using computational techniques to virtually break said user-defined sequence into virtual fragments of length n (n-mers) of defined size,
> providing fragments in vitro by providing fragments of length n (n-mers) of defined size that correspond to said virtual fragments,
> arraying fragments in vitro by arraying said fragments of length n (n-mers) of defined size into groups,
> separating DNA sequence segments temporally in vitro by separating said DNA sequence segments of length n (n-mers) of defined size temporally, and
> assembling groups in vitro by assembling said groups into double-strand DNA molecules of predetermined base-pairs using parallel synthesis, DNA shuffling, and DNA polymerase wherein said step of separating said DNA sequence segments temporally and said step of assembling said groups into double-strand DNA molecules of predetermined base-pairs is accomplished by said DNA sequence segments being added gradually, in an order that is predicted computationally to minimize errors to produce said DNA molecule of user-defined sequence, and
> wherein said step or assembling said groups into double-strand DNA molecules utilizes starting oligos of length n (n-mers) where n is an odd number."

Since the combination of claim elements described above are not found in the Evans reference, the Evans reference does not support a 35 U.S.C. § 102(b) rejection of Appellant's claim 11 and the rejection in Grounds of Rejection #2 should be reversed.

Argument Relating to Grounds of Rejection #3 - The rejection in Grounds of Rejection #3 is respectfully traversed because claims 16 and 17 are not anticipated by the Evans reference. The Evans reference is described above.

The Evans Reference Does Not Anticipate Claims 16 and 17

Appellants will show that Appellant's claims 16 and 17 are not anticipated by the Evans reference. The standard for 35 U.S.C. § 102(a) and 35 U.S.C. § 102(e) rejections is stated in *RCA Corp. v. Applied Digital Systems, Inc,* 221PQ 385, 388 (d. Cir. 1984) "Anticipation is established only when a single prior art reference discloses, either expressly or under principles of inherency, each and every element of a claimed invention." Appellants point out that the following elements of Appellant's claims 16 and 17 are not found in the Evans reference:

> From Parent Claim 11
> "assembling groups in vitro by assembling said groups
> into double-strand DNA molecules of predetermined
> base-pairs using parallel synthesis, DNA shuffling, and

DNA polymerase wherein said step of separating said DNA sequence segments temporally and said step of assembling said groups into double-strand DNA molecules of predetermined base-pairs is accomplished by said DNA sequence segments being added gradually, in an order that is predicted computationally to minimize errors to produce said DNA molecule of user-defined sequence," or

"wherein said step or assembling said groups into double-strand DNA molecules utilizes starting oligos of length n (n-mers) where n is an odd number."

Claim 16
"The method of producing a DNA molecule of user-defined sequence of claim 11 wherein said starting oligos of length n (n-mers) where n is an odd number are starting oligos of length n+1 or n+2."

Claim 17
"The method of producing a DNA molecule of 1-10 kilobases of user-defined sequence from short oligos of length n (n-mers) of claim 11 wherein said multiplicity of DNA sequence segments comprise oligos in multiple reading frames."

Since the elements described above are not found in the Evans reference, the Evans reference does not support a 35 U.S.C. § 102(b) rejection of Appellant's claims 16 and 17 and the rejection in Grounds of Rejection #3 should be reversed.

Appellants point out that the following combination of claim elements specified in Appellant's claims 16 and 17 are not shown by the Evans reference:

Claim 16

"virtually preselecting a multiplicity of DNA sequence segments that will comprise said DNA molecule of user-defined sequence by using computational techniques to virtually break said user-defined sequence into virtual fragments of length n (n-mers) of defined size,
providing fragments in vitro by providing fragments of length n (n-mers) of defined size that correspond to said virtual fragments,
arraying fragments in vitro by arraying said fragments of length n (n-mers) of defined size into groups,
separating DNA sequence segments temporally in vitro by separating said DNA sequence segments of length n (n-mers) of defined size temporally, and
assembling groups in vitro by assembling said groups into double-strand DNA molecules of predetermined base-pairs using parallel synthesis, DNA shuffling, and DNA polymerase wherein said step of separating said DNA sequence segments temporally and said step of assembling said groups into double-strand DNA molecules of predetermined base-pairs is accomplished by said DNA sequence segments being added gradually, in an order that is predicted computationally to minimize errors to produce said DNA molecule of user-defined sequence, and
wherein said step or assembling said groups into double-strand DNA molecules utilizes starting oligos of length n (n-mers) where n is an odd number,
wherein said starting oligos of length n (n-mers) where n is an odd number are starting oligos of length n+1 or n+2."

Claim 17

"virtually preselecting a multiplicity of DNA sequence segments that will comprise said DNA molecule of user-defined sequence by using computational techniques to virtually break said user-defined sequence into virtual fragments of length n (n-mers) of defined size,

providing fragments in vitro by providing fragments of length n (n-mers) of defined size that correspond to said virtual fragments,
arraying fragments in vitro by arraying said fragments of length n (n-mers) of defined size into groups,
separating DNA sequence segments temporally in vitro by separating said DNA sequence segments of length n (n-mers) of defined size temporally, and
assembling groups in vitro by assembling said groups into double-strand DNA molecules of predetermined base-pairs using parallel synthesis, DNA shuffling, and DNA polymerase wherein said step of separating said DNA sequence segments temporally and said step of assembling said groups into double-strand DNA molecules of predetermined base-pairs is accomplished by said DNA sequence segments being added gradually, in an order that is predicted computationally to minimize errors to produce said DNA molecule of user-defined sequence, and
wherein said step or assembling said groups into double-strand DNA molecules utilizes starting oligos of length n (n-mers) where n is an odd number,
wherein said multiplicity of DNA sequence segments comprise oligos in multiple reading frames."

Since the combination of claim elements described above are not found in the Evans reference, the Evans reference does not support a 35 U.S.C. § 102(a) and 35 U.S.C. § 102(e) rejection of Appellant's claims 16 and 17 and the rejection in Grounds of Rejection #3 should be reversed.

Argument Relating to Grounds of Rejection #4 - The rejection in Grounds of Rejection #4 is respectfully traversed because claims 11 and 17 are not obvious over the Selifonov reference and the Evans reference. The Evans reference is described above.

The Selifonov Reference

The Selifonov reference is International Patent No. WO 00/42560. The Selifonov system is described in the Selifonov reference specification and illustrated in the Selifonov Fig. 1A reproduced below.

> "A set of flow schematics which provide a general representation of an exemplary process of Directed Evolution (DE) by GAGGS are enclosed (Figs. 1-4). Fig. 1 provides an example decision making process from an idea of a desired property to selection of a genetic algorithm. Figure 2 provides a directed evolution decision tree from selection of the genetic algorithm to a refined library of parental character strings. Figure 3 provides example processing steps from the refined parental library to a raw derivative library of character strings. Figure 4 processes the raw character strings to strings with a desired property."

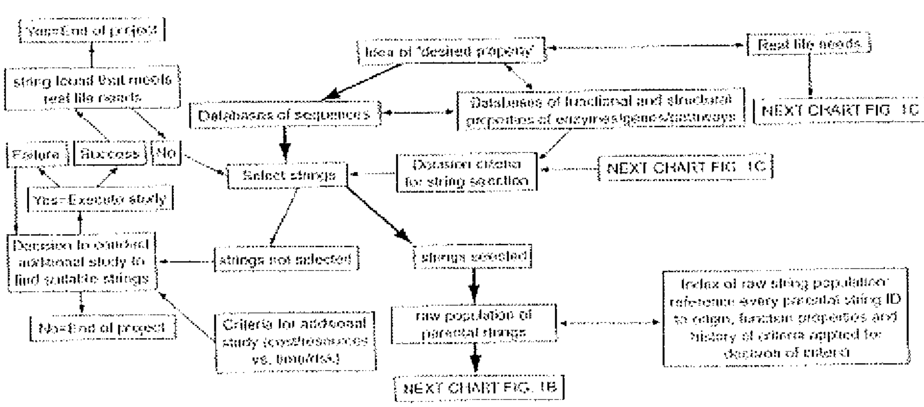

Fig. 1A

"Generally the charts are schematics of arrangements for components, and of process decision tree structures. It is apparent that many modifications of this particular arrangement for DEGAGGS, e.g., as set forth herein, can be developed and practiced. Certain quality control modules and links, as well as most of the genenic artificial neural network learning components are omitted for clarity, but will be apparent to one of skill. The charts are in a continuous arrangement, each connectable head-to tail. Additional material and implementation of individual GO modules, and many arrangements of GOs in working sequences and trees, as used in GAGGS, are available in various software packages. Suitable references describing exemplar existing software are found, e.g., at http://www.aic.nrl.navy.mil/galist/ and at http://www.cs.purdue.edu/ coast/archive/clife/FAQ/www/Q20-2.htm. It will be apparent that many of the decision steps represented in Figs. 1-4 are performed most easily with the assistance of a computer, using one or more software program to facilitate selection/ decision processes."

Prima Facie Case of Obviousness Has Not Been Established

The Examiner bears the initial burden of factually supporting a *prima facie* conclusion of obviousness (M.P.E.P. Section 2142). Three basic criteria must be met in order for the Examiner to establish a *prima facie* case of obviousness.

Criterion 1 - The prior art reference (or reference when combined) must teach or suggest all the claim limitations.

Criterion 2 - There must be a reasonable expectation of success with the proposed combination.

Criterion 3 - The Examiner must follow the "Examination Guidelines for Determining Obviousness in Light of the Supreme Court's KSR v.

Teleflex Decision" published October 10, 2007. These guidelines include the requirement that the Examiner provide reasons for modifying the references to produce the proposed combination.

Criterion 1 - References Do Not Teach All Claim Limitations

The criterion that prior art reference must teach or suggest all the claim limitations has not been met by the Final Rejection. For example, the Selifonov reference and the Evans reference fail to disclose the following claim limitations of claims 11 and 17:

> Claim 11
> "assembling groups in vitro by assembling said groups into double-strand DNA molecules of predetermined base-pairs using parallel synthesis, DNA shuffling, and DNA polymerase wherein said step of separating said DNA sequence segments temporally and said step of assembling said groups into double-strand DNA molecules of predetermined base-pairs is accomplished by said DNA sequence segments being added gradually, in an order that is predicted computationally to minimize errors to produce said DNA molecule of user-defined sequence," or
> "wherein said step or assembling said groups into double-strand DNA molecules utilizes starting oligos of length n (n-mers) where n is an odd number."
>
> Claim 17
> "The method of producing a DNA molecule of 1-10 kilobases of user-defined sequence from short oligos of length n (n-mers) of claim 11 wherein said multiplicity of DNA sequence segments comprise oligos in multiple reading frames."

The Selifonov reference and the Evans reference fail to disclose Appellants' claim limitations identified above. Since these elements and claim limitations are missing from the Selifonov reference and the Evans reference, the rejection in Grounds of Rejection #4 does not meet Criterion 1 of factually supporting a *prima facie* conclusion of obviousness and the rejection in the Grounds of Rejection #4 should be reversed.

Appellants point out that the following combination of claim elements specified in Appellant's claims 11 and 17 are not shown by the Evans reference:

> Claim 11
> "virtually preselecting a multiplicity of DNA sequence segments that will comprise said DNA molecule of user-defined sequence by using computational techniques to virtually break said user-defined sequence into virtual fragments of length n (n-mers) of defined size,
> providing fragments in vitro by providing fragments of length n (n-mers) of defined size that correspond to said virtual fragments,
> arraying fragments in vitro by arraying said fragments of length n (n-mers) of defined size into groups,
> separating DNA sequence segments temporally in vitro by separating said DNA sequence segments of length n (n-mers) of defined size temporally, and
> assembling groups in vitro by assembling said groups into double-strand DNA molecules of predetermined base-pairs using parallel synthesis, DNA shuffling, and DNA polymerase wherein said step of separating said DNA sequence segments temporally and said step of assembling said groups into double-strand DNA molecules of predetermined base-pairs is accomplished by said DNA sequence segments being added gradually, in an order that

is predicted computationally to minimize errors to produce said DNA molecule of user-defined sequence, and

"wherein said step or assembling said groups into double-strand DNA molecules utilizes starting oligos of length n (n-mers) where n is an odd number."

Claim 17

"virtually preselecting a multiplicity of DNA sequence segments that will comprise said DNA molecule of user-defined sequence by using computational techniques to virtually break said user-defined sequence into virtual fragments of length n (n-mers) of defined size,

providing fragments in vitro by providing fragments of length n (n-mers) of defined size that correspond to said virtual fragments,

arraying fragments in vitro by arraying said fragments of length n (n-mers) of defined size into groups,

separating DNA sequence segments temporally in vitro by separating said DNA sequence segments of length n (n-mers) of defined size temporally, and

assembling groups in vitro by assembling said groups into double-strand DNA molecules of predetermined base-pairs using parallel synthesis, DNA shuffling, and DNA polymerase wherein said step of separating said DNA sequence segments temporally and said step of assembling said groups into double-strand DNA molecules of predetermined base-pairs is accomplished by said DNA sequence segments being added gradually, in an order that is predicted computationally to minimize errors to produce said DNA molecule of user-defined sequence, and

wherein said step or assembling said groups into double-strand DNA molecules utilizes starting oligos of length n (n-mers) where n is an odd number,

The Selifonov reference and the Evans reference fail to disclose Appellants' combination of claim elements identified above. Since these

elements and claim limitations are missing from the Selifonov reference and the Evans reference, the rejection in Grounds of Rejection #4 does not meet Criterion 1 of factually supporting a *prima facie* conclusion of obviousness and the rejection in the Grounds of Rejection #4 should be reversed.

Criterion 3 – No Reasons for Combining the References

The criterion that the Examiner must follow the "Examination Guidelines for Determining Obviousness in Light of the Supreme Court's KSR v. Teleflex Decision" published October 10, 2007" has not been met by the Final Rejection. These guidelines include the requirement that the Examiner provide reasons for combining the references to produce the proposed combination. The Final Rejection does not meet Criterion 3 of factually supporting a *prima facie* conclusion of obviousness and the rejection in the Grounds of Rejection #4 should be reversed.

Argument Relating to Grounds of Rejection #5 - The rejection in Grounds of Rejection #5 is respectfully traversed because claim 16 is not obvious over the Selifonov reference and the Evans reference and the Murphy reference. The Selifonov reference and the Evans reference are described above.

The Murphy Reference

The Murphy reference is U. S. Patent No. 6,994,963 for methods for recombinatorial nucleic acid synthesis. The Murphy reference is described in portions of the Murphy reference specification quoted below.

> "The invention provides a method for producing a nucleic acid, comprising the steps of: (a) annealing at least one primer nucleic acid to at least one template nucleic acid, (b) performing a first extension by extending the primer nucleic acid employing the template nucleic acid to form an extended nucleic acid, (c) cleaving the at least one extended nucleic acid, wherein the cleaved extended nucleic acid comprise a nucleic acid extension ladder; (d) denaturing the extended nucleic acid from the template nucleic acid, (d) annealing the extended nucleic acid to at least a second template nucleic acid, (e) performing at least a second extension by extending the extended nucleic acid employing the second template nucleic acid to form a twice extended nucleic acid, (f) adding at least one chain-terminating agent comprising at least one dideoxynucleotide, a dideoxynucleotide analog or a dideoxynucleotide derivative before or during at least one of the first extension or the second extension, wherein said chain-terminating agent is incorporated into said extended nucleic acid, and (g) modifying or removing the chain-terminating agent from the extended nucleic acid, if a further extension is to be performed."

Prima Facie Case of Obviousness Has Not Been Established

The Examiner bears the initial burden of factually supporting a *prima facie* conclusion of obviousness (M.P.E.P. Section 2142). Three basic criteria must be met in order for the Examiner to establish a *prima facie* case of obviousness.

Criterion 1 - The prior art reference (or reference when combined) must teach or suggest all the claim limitations.

Criterion 2 - There must be a reasonable expectation of success with the proposed combination.

Criterion 3 - The Examiner must follow the "Examination Guidelines for Determining Obviousness in Light of the Supreme Court's KSR v. Teleflex Decision" published October 10, 2007. These guidelines include the requirement that the Examiner provide reasons for modifying the references to produce the proposed combination.

Criterion 1 - References Do Not Teach All Claim Limitations

The criterion that prior art reference must teach or suggest all the claim limitations has not been met by the Final Rejection. For example, the Selifonov reference and the Evans reference and the Murphy reference fail to disclose the following claim limitations of claim 16:

> From Parent Claim 11
> "assembling groups in vitro by assembling said groups into double-strand DNA molecules of predetermined base-pairs using parallel synthesis, DNA shuffling, and DNA polymerase wherein said step of separating said DNA sequence segments temporally and said step of assembling said groups into double-strand DNA molecules of predetermined base-pairs is accomplished by said DNA sequence segments being added gradually, in an order that is predicted computationally to minimize errors to produce said DNA molecule of user-defined sequence," or

"wherein said step or assembling said groups into double-strand DNA molecules utilizes starting oligos of length n (n-mers) where n is an odd number."

Claim 16
"The method of producing a DNA molecule of user-defined sequence of claim 11 wherein said starting oligos of length n (n-mers) where n is an odd number are starting oligos of length n+1 or n+2."

The Selifonov reference and the Evans reference and the Murphy reference fail to disclose Appellants' claim limitations identified above. Since these elements and claim limitations are missing from the Selifonov reference and the Evans reference and the Murphy reference, the rejection in Grounds of Rejection #5 does not meet Criterion 1 of factually supporting a *prima facie* conclusion of obviousness and the rejection in the Grounds of Rejection #5 should be reversed.

Appellants point out that the following combination of claim elements specified in Appellant's claim 16 are not shown by the Selifonov reference and the Evans reference and the Murphy reference:

Claim 16
"virtually preselecting a multiplicity of DNA sequence segments that will comprise said DNA molecule of user-defined sequence by using computational techniques to virtually break said user-defined sequence into virtual fragments of length n (n-mers) of defined size,
providing fragments in vitro by providing fragments of length n (n-mers) of defined size that correspond to said virtual fragments,

arraying fragments in vitro by arraying said fragments of length n (n-mers) of defined size into groups,
separating DNA sequence segments temporally in vitro by separating said DNA sequence segments of length n (n-mers) of defined size temporally, and
assembling groups in vitro by assembling said groups into double-strand DNA molecules of predetermined base-pairs using parallel synthesis, DNA shuffling, and DNA polymerase wherein said step of separating said DNA sequence segments temporally and said step of assembling said groups into double-strand DNA molecules of predetermined base-pairs is accomplished by said DNA sequence segments being added gradually, in an order that is predicted computationally to minimize errors to produce said DNA molecule of user-defined sequence, and
wherein said step or assembling said groups into double-strand DNA molecules utilizes starting oligos of length n (n-mers) where n is an odd number,
wherein said starting oligos of length n (n-mers) where n is an odd number are starting oligos of length n+1 or n+2."

The Selifonov reference and the Evans reference and the Murphy reference fail to disclose Appellants' combination of claim elements identified above. Since these elements and claim limitations are missing from the Selifonov reference and the Evans reference and the Murphy reference, the rejection in Grounds of Rejection #5 does not meet Criterion 1 of factually supporting a *prima facie* conclusion of obviousness and the rejection in the Grounds of Rejection #5 should be reversed.

Criterion 3 – No Reasons for Combining the References

The criterion that the Examiner must follow the "Examination Guidelines for Determining Obviousness in Light of the Supreme Court's KSR v. Teleflex Decision" published October 10, 2007" has not been met by the Final Rejection. These guidelines include the requirement that the Examiner provide reasons for combining the references to produce the proposed combination. The Final Rejection does not meet Criterion 3 of factually supporting a *prima facie* conclusion of obviousness and the rejection in the Grounds of Rejection #5 should be reversed.

SUMMARY

Appellants' have demonstrated that claim 16 complies with the written description requirement of 35 U.S.C. § 112, first paragraph. The rejection of claim 16 on appeal in Grounds of Rejection #1 should be reversed.

Appellants' have demonstrated that the Evans reference does not contain many of Appellants' claim elements of claim 11. Claim 11 is not anticipated by the Evans reference under 35 U.S.C. § 102(b) and the rejection in the Grounds of Rejection #2 should be reversed.

Appellants' have demonstrated that the Evans reference does not contain many of Appellants' claim elements of claims 16 and 17. Claims 16 and 17 are not anticipated by the Evans reference under 35 U.S.C. § 102(a) or 35 U.S.C. § 102(e) and the rejection in the Grounds of Rejection #3 should be reversed.

Appellants' have demonstrated that the Selifonov reference and the Evans reference do not disclose many Appellants' claim limitations of claims 11 and 17. The Final Rejection does not meet the criteria of factually supporting a *prima facie* conclusion of obviousness and the rejection in the Grounds of Rejection #4 should be reversed.

Appellants' have demonstrated that the Evans reference and the Murphy reference do not disclose many Appellants' claim limitations of claim 16. The Final Rejection does not meet the criteria of factually supporting a *prima facie* conclusion of obviousness and the rejection in the Grounds of Rejection #5 should be reversed.

It is respectfully requested that claims 11, 16, and 17 on appeal be allowed.

Respectfully submitted,

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Date: December 17, 2008

VIII. CLAIMS APPENDIX

11. A method of producing a DNA molecule of 1-10 kilobases of user-defined sequence from short oligos of length n (n-mers), comprising the steps of:

virtually preselecting a multiplicity of DNA sequence segments that will comprise said DNA molecule of user-defined sequence by using computational techniques to virtually break said user-defined sequence into virtual fragments of length n (n-mers) of defined size,

providing fragments in vitro by providing fragments of length n (n-mers) of defined size that correspond to said virtual fragments,

arraying fragments in vitro by arraying said fragments of length n (n-mers) of defined size into groups,

separating DNA sequence segments temporally in vitro by separating said DNA sequence segments of length n (n-mers) of defined size temporally, and

assembling groups in vitro by assembling said groups into double-strand DNA molecules of predetermined base-pairs using parallel synthesis, DNA shuffling, and DNA polymerase wherein said step of separating said DNA sequence segments temporally and said step of assembling said groups into double-strand DNA molecules of predetermined base-pairs is

accomplished by said DNA sequence segments being added gradually, in an order that is predicted computationally to minimize errors to produce said DNA molecule of user-defined sequence, and

wherein said step or assembling said groups into double-strand DNA molecules utilizes starting oligos of length n (n-mers) where n is an odd number.

16. The method of producing a DNA molecule of user-defined sequence of claim 11 wherein said starting oligos of length n (n-mers) where n is an odd number are starting oligos of length n+1 or n+2.

17. The method of producing a DNA molecule of 1-10 kilobases of user-defined sequence from short oligos of length n (n-mers) of claim 11 wherein said multiplicity of DNA sequence segments comprise oligos in multiple reading frames.

IX. EVIDENCE APPENDIX

There are no entries in the Evidence Appendix.

X. RELATED PROCEEDINGS APPENDIX

There are no entries in the Related Proceedings Appendix.